

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶ : C12N 15/12, 15/85, 5/10, C12P 21/02, A61K 48/00, C07K 14/575, 14/72, 16/26, G01N 33/53	A1	(11) International Publication Number: WO/96/02829 (43) International Publication Date: 14 November 1996 (14.11.96)
(21) International Application Number: PCT/US96/06609 (22) International Filing Date: 8 May 1996 (08.05.96) (30) Priority Data: 08/437,834 8 May 1995 (08.05.95) US (71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94698 (US). (72) Inventors: GIESSE, Klaus, W.; 1365 - 9th Avenue #6, San Francisco, CA 94122 (US). WILLIAMS, Lewis, T.; 3 Miraflores Lane, Tiburon, CA 94920 (US). (74) Agents: GREEN, Grant, D. et al.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).	(81) Designated States: AU, CA, CN, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	
(54) Title: NUCLEIC ACIDS FOR TREATING OBESITY (57) Abstract A nucleic acid molecule is provided that can be expressed in a host cell to produce a biologically active ob polypeptide that can effectively inhibit food intake and/or weight gain. Vectors and host cells containing the nucleic acid molecule are also provided, as well as methods for producing the ob protein and other ob polypeptides, methods of induction of the production of the ob polypeptides, such as by <i>in vivo</i> or <i>ex vivo</i> gene therapy, and methods for inhibition of food intake and/or weight gain. Further provided are antibodies to the ob polypeptides and methods of using such antibodies, such as for identification or detection of other ob polypeptides or homologs thereof, and for inhibition of ob polypeptide activity. A method for identification, detection, or isolation of an ob receptor is provided as well as methods for production of antibodies to the ob receptor. The antibodies and polypeptides herein can be incorporated in kits for immunoassays. Pharmaceutical compositions containing the ob polypeptide and antibodies to the ob polypeptide or to the ob receptor can be used for administration to animals and humans.		

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a first nucleotide sequence that encodes an expression control sequence and a second nucleotide sequence that encodes an ob polypeptide, wherein the second nucleotide sequence is under regulatory control of the first nucleotide sequence, and the first nucleotide sequence is not naturally associated with the second nucleotide sequence.
2. The nucleic acid molecule of claim 1, further comprising a third nucleotide sequence, wherein the third nucleotide sequence encodes a secretion leader sequence that is sufficient for secretion of the ob polypeptide upon expression of the nucleic acid molecule in a host cell.
3. The nucleic acid molecule of claim 2, wherein the secretion leader sequence is not naturally associated with the ob polypeptide.
4. The nucleic acid molecule of claim 1, wherein the expression control sequence is one selected from the group consisting of a prokaryotic cell promoter and an eukaryotic cell promoter or a viral promoter.
5. An expression vector comprising the nucleic acid molecule of claim 1 and a third nucleotide sequence that encodes a marker.
6. The vector of claim 5, further comprising a fourth nucleotide sequence, wherein the fourth nucleotide sequence encodes a secretion leader sequence that is sufficient for secretion of the ob polypeptide upon expression of the nucleic acid molecule in a host cell.
7. The vector of claim 6, wherein the secretion leader sequence is not naturally associated with the ob polypeptide.

8. The vector of claim 5, wherein the vector comprises a nucleic acid molecule selected from the group consisting of constructs #1122, #1123, #1124, #1130, #1131, #1132, #1127, #1128, #1129, #1150, #1142, #1143, #1144, #1145, and #1147.

9. A host cell comprising the vector of claim 5.

10. The host cell of claim 9, wherein the vector further comprises a third nucleotide sequence, wherein the third nucleotide sequence encodes a secretion leader sequence that is sufficient for secretion of the ob polypeptide upon expression of the nucleic acid molecule in a host cell.

11. The host cell of claim 9, wherein the cell is selected from a group consisting of a prokaryotic cell and an eukaryotic cell.

12. The host cell of claim 9, wherein the cell is a prokaryotic cell, and prokaryotic cell is *Escherichia coli*.

13. The host cell of claim 11, wherein the cell is an eukaryotic cell, and the eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, and a yeast cell.

14. The host cell of claim 13, wherein the eukaryotic cell a mammalian cell and the mammalian cell is a human cell.

15. A method for the production of an ob polypeptide comprising:

- a) providing the nucleic acid molecule of claim 1;
- b) introducing the nucleic acid molecule into a cell that is capable of expressing the ob polypeptide; and
- c) allowing the expression of the ob polypeptide in the cell.

16. A method for production of an ob polypeptide comprising:
 - a) providing the vector of claim 5;
 - b) introducing the vector into a host cell; and
 - c) allowing expression of the ob polypeptide in the cell.
17. A method for production of an ob polypeptide comprising:
 - a) providing the host cell of claim 9; and
 - b) allowing expression of the ob polypeptide in the cell.
18. A method for induction of production of an ob polypeptide in a mammal comprising administering to a mammal the nucleic acid molecule of claim 1.
19. The method of claim 18, wherein the nucleic acid molecule is delivered directly or by viral or non-viral means.
20. A method for induction of production of an ob polypeptide in a mammal comprising administering to the mammal the vector of claim 5.
21. An ob polypeptide produced by the method comprising:
 - a) providing a host cell comprising the nucleic acid molecule of claim 1; and
 - b) allowing the expression of the ob polypeptide.
22. A method for inhibition of weight gain comprising administering a weight-gain-inhibitory amount of the ob polypeptide of claim 21.
23. A method for the inhibition of food intake comprising administering a food-intake-inhibitory amount of the ob polypeptide of claim 21.

24. A method for production of an ob receptor comprising:

- a) providing a labeled ob polypeptide, wherein the ob polypeptide that is labeled is the polypeptide of claim 21;
- b) allowing the labeled ob polypeptide to react with cells or portions or extracts thereof to form a binding pair; and
- c) separating from the binding pair an ob receptor that binds to the labeled ob polypeptide.

25. An antibody comprising an amino acid sequence, wherein the sequence is capable of binding to the ob polypeptide of claim 21 to form a binding pair.

26. The antibody of claim 25, wherein the antibody is a mammalian antibody or a humanized antibody.

27. The antibody of claim 26, wherein the antibody is a murine antibody or a human antibody.

28. A method for identification of an ob polypeptide or an ob polypeptide homolog comprising contacting a labeled antibody with a sample suspected of containing an ob polypeptide or an ob polypeptide homolog to allow formation of a binding pair, and determining the identity of the binding pair, wherein the antibody that is labeled is the antibody of claim 25.

29. A method for production of an antibody to an ob receptor comprising administering to an animal the ob receptor of claim 30, and collecting from the animals either serum containing antibodies or spleen cells for the production of monoclonal antibodies.

30. An ob receptor produced by the method of claim 24.

31. A method for detection of an ob receptor comprising:
 - a) providing a labeled antibody to an ob receptor, wherein antibody that is labeled is the antibody of claim 29;
 - b) allowing the labeled antibody to react with a cell or portions or extracts thereof to allow formation of a binding pair; and
 - c) determining the presence of the binding pair.
32. A method for the detection of an ob polypeptide comprising:
 - a) providing a labeled antibody to an ob polypeptide, wherein the antibody that is labeled is the antibody of claim 25;
 - b) allowing the labeled antibody to react with an ob polypeptide to form a binding pair; and
 - c) determining the presence of the binding pair.
33. A kit for detection of an ob polypeptide comprising the antibody of claim 25.
34. A kit for detection of an ob receptor comprising the antibody of claim 29.
35. A kit for the detection of an ob receptor comprising a labeled ob polypeptide, wherein the polypeptide that is labeled is the polypeptide of claim 21.
36. A kit for the detection of antibodies to an ob polypeptide comprising a labeled ob polypeptide, wherein the polypeptide that is labeled is the polypeptide of claim 21.
37. A pharmaceutical composition comprising the ob polypeptide of claim 21 and a pharmaceutically acceptable carrier.
38. A method of blocking the activity of ob polypeptide in a mammal by administering the antibody of claim 25.